Project Report: Enzymes of Ancient Metabolic Pathways

Project Progress

Since 1933, carbonic anhydrase research has focused on enzymes from mammals (α class) and plants (β class); however, two additional classes (γ and δ) were discovered recently. Cam, from the procaryote *Methanosarcina thermophila*, is the prototype of the γ class and the first carbonic anhydrase to be characterized from either an anaerobic organism or the *Archaea* domain. All enzymes characterized from the four classes have been purified aerobically and are reported to contain a catalytic zinc. Herein, we report the anaerobic reconstitution of apo–Cam with Fe^{2+} which yielded Cam with an effective k_{cat} which exceeded that for the Zn^{2+}–reconstituted enzyme. Mössbauer spectroscopy showed that the Fe^{2+}–reconstituted enzyme contained high–spin Fe^{2+} that when oxidized to Fe^{+3} inactivated the enzyme. Reconstitution with Fe^{+3} was unsuccessful. Reconstitution with Cu^{2+}, Mn^{2+}, Ni^{2+}, or Cd^{2+} yielded enzymes with effective k_{cat} values that were 10% or less than the value for the Zn^{2+}–reconstituted Cam. Cam produced in *Escherichia coli* and purified anaerobically contained iron with effective k_{cat} and k_{cat}/Km values exceeding the values for Zn^{2+}–reconstituted Cam. The results identify a previously unrecognized biological function for iron.

Roadmap Objectives

- ***Objective No. 1.1:*** Models of formation and evolution of habitable planets
- ***Objective No. 3.2:*** Origins and evolution of functional biomolecules
- ***Objective No. 3.3:*** Origins of energy transduction
- ***Objective No. 4.1:*** Earth's early biosphere
- ***Objective No. 4.2:*** Foundations of complex life
- ***Objective No. 5.1:*** Environment–dependent, molecular evolution in microorganisms
- ***Objective No. 5.3:*** Biochemical adaptation to extreme environments
- ***Objective No. 6.1:*** Environmental changes and the cycling of elements by the biota, communities, and ecosystems
- ***Objective No. 6.2:*** Adaptation and evolution of life beyond Earth